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1 **SEMIPERMEABLE MEMBRANE DEVICES LINK SITE-SPECIFIC**
2 **CONTAMINANTS TO EFFECTS: PART II – A COMPARISON OF LINGERING**
3 **EXXON VALDEZ OIL WITH OTHER POTENTIAL SOURCES OF CYP1A**
4 **INDUCERS IN PRINCE WILLIAM SOUND, ALASKA**

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23

24

25 **Abstract**

26

27 We deployed semi-permeable membrane devices (SPMDs) on beaches for 28 days at 53
28 sites in Prince William Sound (PWS), Alaska, to evaluate the induction potential from
29 suspected sources of cytochrome P450 1A (CYP1A)-inducing contaminants. Sites were
30 selected to assess known point sources, or were chosen randomly to evaluate the region-
31 wide sources. After deployment, SPMD extracts were analyzed chemically for persistent
32 organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAH). These results
33 were compared with hepatic CYP1A enzyme activity of juvenile rainbow trout injected
34 with the same extracts prior to clean-up for the chemical analyses. Increased CYP1A
35 activity was strongly associated with PAH concentrations in extracts, especially chrysene
36 homologues but was not associated with POPs. The only apparent sources of chrysene
37 homologues were lingering oil from *Exxon Valdez*, asphalt and bunker fuels released
38 from storage tanks during the 1964 Alaska earthquake, creosote leaching from numerous
39 pilings at one site, and PAH-contaminated sediments at Cordova Harbor. Our results
40 indicate that PWS is remarkably free of pollution from PAH when nearby sources are
41 absent as well as from pesticides and PCBs generally.

42

43 **Keywords:** Pollution monitoring, oil spills, sources, PAH, PCB, SPMD

44

45 **1. Introduction**

46

47 Oil from the T/V *Exxon Valdez* (EVO) has been surprisingly persistent on some
48 beaches in western Prince William Sound (PWS), after deposition following the spill in
49 1989. Estimated loss rates of $< 4\% \text{ yr}^{-1}$ for the buried oil imply persistence for decades
50 (Short et al. 2007), presenting a potentially long-term reservoir of bioaffective
51 contaminants to wildlife. However, distinguishing any long term biological effects of
52 this oil from those of other contaminant sources is highly problematic.

53
54 Several species that interact with the intertidal zone in the spill-impacted part of
55 PWS have shown prolonged expression of cytochrome P450 1A (CYP1A), a substrate-
56 inducible enzyme that is a sensitive biomarker of exposure to planar contaminants such
57 as polycyclic aromatic hydrocarbons (PAH), certain polychlorinated biphenyls (PCBs)
58 and other persistent organic pollutants (POPs). Expression of CYP1A in PWS nearshore
59 vertebrates such as sea otters (*Enhydra lutris*) harlequin ducks (*Histrionicus histrionicus*)
60 and crescent gunnels (*Pholis laeta*) has been highest in animals taken from locations
61 where EVO has been most persistent, and has appeared to decrease as the oil dispersed
62 (Bodkin et al. 2002, Jewett et al. 2002, Trust et al. 2000). These patterns suggest that
63 exposure to remaining EVO, a known CYP1A inducer (Woodin et al., 1997), may be the
64 source of the CYP1A induction observed in these animals.

65
66 Whether the EVO remaining on PWS beaches is sufficiently bioavailable to
67 induce CYP1A in wildlife is a matter of some controversy. Once hardened into highly
68 viscous tar mats and asphalt pavements, the resistance to mass transfer of PAH is so great
69 that loss rates of PAH may become extremely low. However, sea otters and ducks can

70 disturb sediments while foraging (Kvitek and Oliver 1992; Calkins 1978), and any
71 subsurface oil encountered could contaminate their pelage or plumage. Oil ingested
72 during subsequent preening (Bodkin et al. 2002, Trust et al. 2000) provides an exposure
73 pathway for eliciting CYP1A induction and potentially toxic effects (Schwartz et al.
74 2004).

75
76 Evidence of CYP1A induction alone is not sufficient to identify the source of the
77 inducing agent when numerous potential sources may be present (Short and Springman
78 2006). In PWS, several sources of potential CYP1A inducers besides EVO must be
79 considered. Putative sources include atmospheric transport of POPs (Stern et al. 1997),
80 PAH associated with erosion of terrestrial hydrocarbon source rock outcrops transported
81 hydraulically to the northern Gulf of Alaska and then by the Alaska Coastal Current to
82 PWS where they are deposited on the seafloor (Page et al. 1996, Short et al. 2007), POPs
83 associated with adult salmon returning to PWS to spawn (Krümmel et al. 2003, Ewald et
84 al. 1998), small spills of diesel and bilge oils from marine vessels, residues of asphalt and
85 bunker fuels derived from the Monterey Formation in California and released from
86 storage tanks in PWS that ruptured during the 1964 Alaska earthquake (Kvenvolden et al.
87 1995), and operations associated with continuing industrial activity in PWS such as
88 salmon hatcheries. Resolving contributions from these prospective sources to observed
89 CYP1A responses in biota presents a considerable challenge.

90
91 One promising approach for evaluating the CYP1A induction potential of a
92 contaminant source involves injecting fish with contaminants accumulated by semi-

93 permeable membrane devices (SPMDs) deployed at locations strongly affected by the
94 candidate source (Springman et al. 2008). This approach does not require knowledge of
95 the chemical identity of the suspected contaminant, although analysis may provide this.
96 Rather, it examines the biomarker response from the bioavailable complex mixture
97 without selective filtration if the accumulated contaminants are injected without cleanup.
98 At a minimum, prospective contaminant sources that are not capable of eliciting CYP1A
99 responses in biota can be identified at high confidence when the injected contaminants
100 fail to elicit a response, provided the amount of contaminant injected is comparable with
101 those likely to be accumulated through passive equilibrium partitioning by the same
102 organisms exposed in the field.

103

104 Our objectives in this study are to distinguish which contaminant sources in PWS
105 are capable of eliciting CYP1A activity in dosed fish and to identify which compounds of
106 the sampled complex mixture are most responsible. To accomplish this, we deployed
107 SPMDs at a variety of locations that included all the prospective contaminant sources
108 mentioned above. In Part I of this study, we measured the CYP1A induction potential of
109 contaminants extracted from deployed SPMDs and injected without clean-up into
110 juvenile rainbow trout (*Oncorhynchus mykiss*, Springman et al. 2008). Here we present
111 chemical analysis results of extracts from SPMDs deployed to evaluate the compounds
112 most likely responsible for induction, and of additional SPMDs deployed to better
113 characterize contaminant sources and distributions in PWS.

114

115 **2. Methods**

116

117 *2.1 Study Sites*

118

119 We deployed SPMDs at 53 sites within PWS, including known and suspected
120 contaminant source sites and sites selected at random to characterize the pollution burden
121 of the Sound (Fig. 1). Known contaminant source sites include boat harbors at Cordova
122 (site 1, Table 1) and Chenega (site 2), patches of Alaska North Slope (ANS) oil
123 remaining from the 1989 *Exxon Valdez* oil spill (sites 3 - 7) or of Monterey Formation
124 bunker fuels released from ruptured fuel tanks at industrial “human activity” (HA) sites
125 during the 1964 Alaska earthquake (sites 8 - 12), and salmon hatcheries (sites 13 - 17).
126 Salmon streams (sites 18 - 22) are suspected as a source of contaminants released from
127 decomposing carcasses of mainly pink salmon (*Oncorhynchus gorbuscha*) following
128 spawning. One stream from each of the five largest Alaska Department of Fish and
129 Game districts was selected based on three criteria: 1) large returns of salmon during our
130 study period, 2) small stream channels and narrow deltas where fish (with putative
131 contaminants) would be concentrated, and 3) accessibility via float plane.

132

133 To characterize region-wide, non-point contaminant sources, 30 sites were
134 distributed at random among 5 sectors partitioning the Sound (depicted in Fig. 1). The
135 number of sites within each sector was proportional to the total sector shoreline length
136 (sites 23 – 52). This stratification reduced site clustering that could result from un-
137 restricted random selection, ensuring site dispersion throughout the sound. Additionally,
138 to better evaluate PAH availability, one SPMD was located at a long-term monitoring

139 station (Karinen et al., 1993) in Constantine Harbor (site 53). At this site, PAH
140 associated with hydrocarbon-rich rocks eroded from geologic sources east of PWS have
141 an intertidal sediment concentration of 561 ± 15.2 ng/g (\pm standard error here and
142 following) including the same suites of 2- to 4-ring PAH as are typically found in crude
143 oils (Short and Babcock 1996). Finally, one site was located in Graves Harbor (site 54),
144 a remote site in southeast Alaska ~ 300 km east of PWS and upcurrent of the geologic
145 hydrocarbon sources east of PWS to serve as a regional control.

146

147 The ANS sites (3 – 7) correspond with the following respective beach segment
148 identifiers that are used extensively in the literature on the *Exxon Valdez* oil spill,
149 including Part I of this study (Springman et al. 2008): DI067A, EL056C, KN109A,
150 KN114A, and KN136A. These sites are on Disk Island, Eleanor Island and Knight
151 Island.

152

153 Random site locations were determined by considering the shoreline within a
154 sector as a continuous line of total length L , generating a random number r between 0 and
155 1, and finding the location coordinates associated with the product ($r \cdot L$). The SPMD was
156 deployed at the nearest suitable location to the calculated coordinates.

157

158 Additional local control sites were selected for the ANS and HA sites to evaluate
159 the spatial extent of contaminants from these sources. At the ANS sites, SPMDs were
160 deployed within patches of beach that contained obvious subsurface oil, to promote
161 accumulation of dissolved oil components. Associated local control deployments were

162 on apparently un-oiled beaches within 0.5 km of the oiled SPMD deployment location.
163 At the HA sites, SPMDs were deployed within patches of surface asphalt (4 sites), or
164 subsurface oil at one site (site 9), again to promote accumulation of dissolved oil
165 components, and local control SPMDs were deployed along shore on un-oiled portions of
166 the same beach, within 100 m either side of the oil deposit.

167

168 *2.2 SPMD Deployment and Processing*

169

170 All the SPMDs were deployed at mid-tide height on beaches, in one of 2 types of
171 canisters. The SPMDs were obtained from Environmental Sampling Technologies (EST,
172 St. Joseph, MO), and were usually deployed in cylindrical cages 30 cm long, each
173 containing 5 SPMDs woven on stainless steel carriers stacked within the cage. Only 1
174 SPMD was deployed at the local HA control locations and at 21 of the random sites,
175 contained within a canister housing 6 cm long. Each SPMD was 91.4 cm long and 2.5
176 cm wide, and contained 1 ml of ultra-high purity triolein. Canisters containing 5 SPMDs
177 were used to increase the amount of accumulated contaminants available for chemical
178 analysis and for injection into juvenile rainbow trout that were assayed for CYP1A
179 induction (see Springman et al. 2008 for details), while those containing only 1 SPMD
180 were used to extend the geographic range of contaminant surveillance and were only
181 analyzed chemically.

182

183 At each deployment site, the cages were half-buried in pits excavated on
184 shorelines at the mid-tide level of each site, with the other half exposed to the

185 atmosphere. After placement in a pit, the excavated material was replaced to the level of
186 the beach surface, surrounding, but not covering, the cage. The grain sizes of sediments
187 on most PWS beaches range from pebbles to boulders, with relatively little mass
188 associated with sand and finer-grained sediments that could pass through the 2 mm
189 diameter of the SPMD cage perforations (Hayes and Michel 1999). Hence, little
190 sediment accumulated within the cages while deployed, and in no case more than
191 partially covered the bottom-most SPMD in the cage. This deployment arrangement
192 therefore exposed SPMDs to the atmosphere at lower tides, to surface seawater at higher
193 tides, and to mainly interstitial water flow within the upper 15 cm of beach for the larger
194 canisters, while minimizing biofouling and direct contact with beach sediments. Field
195 blank cages were briefly exposed to the atmosphere during some of the deployments.
196 Cages were deployed for 27 – 28 days, and then retrieved for dialysis of accumulated
197 contaminants.

199 Contaminants accumulated by the SPMDs were recovered by dialysis into hexane
200 at EST labs as described in Huckins et al. (2000). The dialysates from all SPMDs within
201 a deployment canister were combined to create a composite sample. Following
202 concentration of the dialysates to ~1 ml hexane, samples were partitioned into
203 subsamples for analysis. For samples derived from 5 SPMDs, the sample was partitioned
204 into 3 aliquots for chemical analyses each containing 5% of the sample, and 2 more
205 aliquots of 42.5% each for injection into juvenile trout for determination of CYP1A
206 activity. One each of the aliquots for injection and for chemical analysis was archived in
207 the event of accidental losses. For samples derived from 1 SPMD, the sample was split

208 into 2 aliquots for chemical analysis of PAH and POPs with no sample spared for
209 archives. This arrangement produced twice as much contaminant mass available for
210 chemical analysis from the single-SPMD deployments compared with the deployments
211 involving 5 SPMDs (0.5 vs. $(5 \times 0.05 = 0.25)$) assuming equivalent deployment
212 conditions and performance, resulting in expected environmental detection limits for the
213 single-SPMD deployments to be lower by a factor of 2.

214

215 *2.3 Sample Analysis*

216

217 The sample extracts were analyzed chemically for suites of 2- to 5-ring PAH
218 (including alkyl-substituted and dibenzothiophene homologues, the latter more accurately
219 denoted as polycyclic aromatic compounds but included here as PAH), selected legacy
220 pesticides and selected PCBs listed in Table 1. The PAH were analyzed in sets of twelve
221 plus one method blank, one spiked blank, and two reference samples at ABL following
222 the method described in Short et al. (1996). Briefly, the hexane aliquot for PAH analysis
223 was purified by 20 g:5 g silica:alumina column chromatography and gel permeation
224 HPLC, following addition of a suite of perdeuterated PAH used as internal standards.
225 Purified PAH were measured by gas chromatography/mass spectrometry (GCMS)
226 operated in the selected ion monitoring mode, and were quantified on the basis of a 5-
227 point calibration curve of the most similar PAH standard available.

228

229 The POPs were analyzed in sets of four to twelve SPMD samples plus one
230 method blank and one spiked method blank. Method blanks were prepared by adding 0.5

231 ml of dichloromethane to a 2-ml vial. Spiked method blanks were prepared by adding
232 0.5 ml of dichloromethane and a spiking solution of analytes to a 2-ml vial; PCBs were
233 spiked at approximately 0.3-1.3 ng sample⁻¹ and pesticides were spiked at approximately
234 6 ng sample⁻¹. An internal standard (PCB 103) was added to each method blank, spiked
235 blank and SPMD sample in its original 2-ml vial. PCBs are identified here according to
236 the scheme presented by Ballschmiter et al. (1992)

237

238 Samples were cleaned up by size-exclusion high-performance liquid
239 chromatography using a 350-mm x 21.2-mm Envirosep ABC[®] preparatory column and a
240 60-mm x 21.2-mm Envirosep ABC[®] guard column, (Phenomenex, Inc., Torrance, CA),
241 with dichloromethane as the mobile phase (Sloan et al. 2005). Extracts containing low
242 amounts of PAH etc. were also cleaned up by gravity-flow silica/alumina
243 chromatography using columns packed with 20 cc of silica gel on top of 10 cc of alumina
244 wetted with dichloromethane, and the samples were eluted with 45 ml of
245 dichloromethane (Sloan et al. 2005). Extracts containing high amounts of PAH etc. were
246 cleaned up by gravity-flow acidic/basic silica chromatography. Columns were packed
247 with 5 cc of neutral silica gel (lower layer), 5 cc of KOH impregnated silica gel (middle
248 layer), and 15 cc of 40% (w/w) sulfuric acidic impregnated silica gel (upper layer) wetted
249 with 60 ml of 1:1 dichloromethane:hexane. The samples were eluted with 45 ml of 1:1
250 dichloromethane:hexane 40 minutes after being loaded on the column to allow reaction
251 with the acidic silica (Krahn et al. 1994, modified; see also Sloan et al. 2005 for further
252 details).

253

254 All samples were analyzed by GCMS in selected-ion monitoring mode using a
255 60-m x 0.25-mm, 0.25- μ m film thickness DB-5[®] GC column (J & W Scientific, Folsom,
256 CA) and a 10-m x 0.53-mm deactivated fused-silica guard column, with cool on-column
257 injection and helium carrier gas (Sloan et al. 2005). The concentrations of analytes were
258 calculated based on the internal standard and point-to-point (also known as piece-wise
259 linear) calibration using six concentrations of calibration standards ranging from
260 approximately 0.001 to 0.1 ng μ l⁻¹ for each analyte. The lower limit of quantitation
261 (LOQ) was reported when the GCMS response for that analyte in that sample was below
262 the GCMS calibration range. LOQs were calculated individually by analyte and by
263 sample (Sloan et al. 2004).

264

265 Induction of CYP1A in juvenile trout livers following injection of sample aliquots
266 was evaluated by the ethoxyresorufin-*O*-deethylase (EROD) assay, based on an original
267 method by Pohl and Fouts (1980) and modified for a microplate spectrofluorometer
268 (Hodson et al. 1996). Molar specific activity was reported as picomole resorufin per mg
269 protein per minute (pmol mg⁻¹ min⁻¹). Details of the exposure conditions and assay
270 methods are given in Springman et al. (2008).

271

272 The oil in the patches at the ANS sites had previously been confirmed as remnants
273 from the 1989 Exxon Valdez oil spill (Short et al. 2004). The Monterey Formation
274 source of the oil at the HA sites was confirmed on the basis of tricyclic and tetracyclic
275 terpane analysis as described by Kvenvolden et al. (1995).

276

277 2.4 Data Analysis

278

279 Concentrations of PAH in the SPMDs are presented as $\text{ng g}_{\text{SPMD}}^{-1}$, with three
 280 figures significant. To facilitate comparison among samples, individual PAH
 281 concentrations were normalized to the total PAH (TPAH) concentration of the sample.

282

283 A rough approximation of ambient dissolved PAH concentrations may be inferred
 284 from concentrations measured in SPMDs. Sundberg et al. (2005) estimate dissolved
 285 concentrations corresponding with contaminant concentrations measured in SPMDs as:

$$286 \quad C_w = C_{\text{SPMD}} \cdot k_{I(s)}^{-1} \cdot t^{-1} \quad (1)$$

287 where C_w and C_{SPMD} are the contaminant concentrations in water and the SPMD, $k_{I(s)}$ is
 288 the sampling uptake rate constant of the SPMD for the contaminant in $\text{l d}^{-1} \text{g}_{\text{SPMD}}^{-1}$, and t
 289 is the exposure time of the SPMD to the sampled medium. Luellen and Shea (2002)
 290 present estimates of PAH uptake rates as device sampling rates R_S (in l d^{-1}), where $R_S =$
 291 $m \cdot k_{I(s)}$, and m is the mass of the SPMD. The average value of R_S for the 2 – 4 ring PAH
 292 listed by Luellen and Shea (2002) is 4.4 l d^{-1} for SPMDs identical with ours, implying
 293 values of $k_{I(s)}$ near $1 \text{ l d}^{-1} \text{g}_{\text{SPMD}}^{-1}$, and $k_{I(s)}$ estimated for PCBs are also near $1 \text{ l d}^{-1} \text{g}_{\text{SPMD}}^{-1}$
 294 (Meadows et al. 1998). Assuming an effective exposure time of 14 d (because the
 295 SPMDs were submerged half the time during 28 days and neglecting PAH absorbed from
 296 the atmosphere) implies aqueous TPAH concentrations on the order of $C_w = C_{\text{SPMD}} \cdot (14$
 297 $\text{d})^{-1} \text{ ng l}^{-1}$ when C_{SPMD} is given as $\text{ng g}_{\text{SPMD}}^{-1}$. Given the numerous assumptions
 298 involved, including neglect of partitioning with particulate organic matter, constant
 299 uptake rates during the exposure period and neglect of increased uptake from direct

300 contact with sediments infiltrating the deployment cages (Booij et al. 2003), this
301 approximation is probably only accurate to within a factor of 3 – 5, which suffices to
302 indicate the order of magnitude of the mean aqueous PAH concentrations.

303

304 **3. Results and Discussion**

305

306 *3.1 Boat Harbors and Anchorages*

307

308 The entrance to Cordova Harbor is constricted, promoting retention of
309 contaminants released from the numerous potential sources within it, and leading to
310 substantial accumulation of PAH by the SPMD deployed there. The TPAH concentration
311 of the SPMD was 73,700 ng g⁻¹, equivalent to ambient PAH concentrations on the order
312 of 5 µg l⁻¹ (eq. 1). Phenanthrenes/anthracenes and fluoranthenes/pyrenes were the most
313 abundant homologous series, declining in abundance with increasing alkyl substitution
314 within each series (Fig. 2A). This PAH distribution pattern reflects the composite input
315 from multiple sources, including spilled fuels and lubricants, and combustion exhaust
316 products from inboard and outboard motors.

317

318 The much smaller and less confined harbor at Chenega results in considerably
319 lower PAH concentrations compared with Cordova harbor. The TPAH concentration in
320 the Chenega SPMD, 1,690 ng g⁻¹, was less than 2% of the concentration accumulated
321 during the Cordova harbor deployment, and equivalent to dissolved concentrations on the
322 order of ~121 ng l⁻¹. The PAH distribution pattern from Chenega resembled that of the

323 Cordova deployment, with phenanthrenes/anthracenes and fluoranthenes/pyrenes the
324 most abundant homologous series, and with declining abundance with increasing alkyl
325 substitution within each series (Fig. 2B). Nearly identical patterns were evident at the
326 two random sites where TPAH were greatest, in Sawmill Bay (site 26, about 3 km from
327 the Chenega harbor site and within the same embayment), and at Windy Bay (site 44, an
328 embayment on the eastern side of PWS enclosing a commercial oyster farm), where the
329 TPAH concentrations were 374 and 442 ng g⁻¹, respectively (Table 2). These SPMD
330 concentrations imply that embayments that are more open than the harbor at Cordova
331 have TPAH concentrations on the order of 30 ng l⁻¹, likely from outboard motors and
332 other small boat activity.

333

334 The concentrations of total PCBs and pesticides in the SPMDs indicate that these
335 contaminants rarely exceeded 1 ng l⁻¹ in ambient seawater. At 32.9 ng g⁻¹, the total PCB
336 concentration in the SPMD deployed at Chenega harbor was among the highest of any
337 found during this study (41.8 ng g⁻¹ at nearby Sawmill Bay, site 26; Table 2). At
338 Cordova harbor the SPMD concentration was only 6.31 ng g⁻¹. Elsewhere, 90% of the
339 SPMD concentrations were below 7 ng g⁻¹ (median = 2.67 ng g⁻¹, *N* = 89). These
340 concentrations substantially exceeded those of the laboratory blanks (range: <LOQ – 0.40
341 ng g⁻¹, *N* = 4) but not the field blanks (range: 2.00 – 16.3 ng g⁻¹, *N* = 8). The distribution
342 of relative PCB concentrations was nearly identical among all the samples analyzed,
343 including field blanks, with almost all the PCBs present within the congener range 17 –
344 153 inclusive (Fig. 3). This distribution pattern is similar to that evident in alpine lakes
345 of Europe (Fernandez and Grimalt 2003, Van Drooge et al. 2004) or in open waters of the

346 Baltic Sea (Sundberg et al. 2005), indicating surface water absorption of gas-phase PCBs
347 from the atmosphere. These concentrations imply total aqueous PCB concentrations
348 were below $\sim 1 \text{ ng l}^{-1}$ throughout PWS, except near Sawmill Bay where concentrations
349 approach 3 ng l^{-1} . As noted by Sundberg et al. (2005), these part per trillion
350 concentrations are unlikely to stimulate CYP1A induction in exposed biota.

351

352 Pesticide concentrations in the deployed SPMDs were at least as low as those of
353 the PCBs, and were within the range of concentrations found in field and dialysis blanks.
354 The summed concentrations of the pesticides listed in Table 1 ranged from $<\text{LOQ} - 5.52$
355 ng g^{-1} ($N = 87$), compared with concentrations in field blanks ranging from $0.08 - 4.73 \text{ ng}$
356 g^{-1} ($N = 8$), and in dialysis blanks ranging from $0.03 - 2.92 \text{ ng g}^{-1}$. The similarity of
357 these concentrations suggests that the detected pesticides were trace level residues
358 incorporated during SPMD manufacture or processing, rather than an indication of
359 ambient environmental concentrations in PWS. Because the concentrations of PCBs and
360 pesticides were uniformly below thresholds likely to induce CYP1A in biota throughout
361 PWS, we dismissed them as material factors and focused on PAH at the other sites
362 considered in more detail below.

363

364 Injection of the SPMD extracts from the Cordova harbor SPMD elicited a strong
365 2 d EROD response of $36 \text{ pmol mg}^{-1} \text{ min}^{-1}$, compared with a weak response from the
366 Chenega harbor SPMD ($5.8 \text{ pmol mg}^{-1} \text{ min}^{-1}$; Table 2). The 7 d EROD response from
367 Chenega was $2.97 \text{ pmol mg}^{-1} \text{ min}^{-1}$, and all these responses were significantly ($P < 0.001$)
368 greater than those from control and un-contaminated sites

369

370 *3.2 ANS Sites*

371

372 The SPMDs deployed at the oiled ANS sites (sites 3 – 7, Table 2) consistently
373 accumulated substantial PAH burdens. The TPAH concentration in the SPMDs ranged
374 from 8,920 – 263,000 ng g⁻¹, equivalent to aqueous concentrations of 640 – 19,000 ng l⁻¹
375 (eq. 1). These aqueous concentration estimates are comparable with direct measurements
376 of dissolved-phase PAH in interstitial water that drained similar ANS oil patches in 2002,
377 which ranged from 76 – 4,600 ng l⁻¹ (average = 1,200 ng l⁻¹; Payne et al. 2005). The
378 distribution of PAH homologues showed the characteristic preponderance of alkyl-
379 substituted PAH (Fig. 4A), which followed a weathering pattern diagnostic of ANS oil
380 (Short and Heintz 1997), with chrysene homologues accounting for 5 – 9% of the TPAH.
381 Accumulated PAH in replicate SPMD deployments at 2 of the 3 replicated sites agreed
382 within a factor of 2, but at site 3 (at Disk Island), the PAH concentration in one replicate
383 was ~ 19-fold greater than the other. The Disk Island replicates were separated by 25 m
384 in an extensive deposit of peat, and the low-PAH replicate was located in a surface
385 stream above the peat layer that continually flushed the SPMD, which may have
386 suppressed PAH accumulation. Previous studies have shown that humic substances
387 (including peat) increase the CYP1A induction of oil when the two are co-located,
388 possibly by increasing the solubility of petroleum hydrocarbons (Matsuo et al. 2006).
389 The stream at the Disk Island low PAH replicate transported the humic substances from
390 the deployment site, and these results were reflected in the EROD activity elicited by
391 extracts from these sites, particularly at 7 d.

392

393 The injected aliquots of the SPMD extracts from the oiled ANS sites consistently
394 elicited strong 2 d EROD responses, and often these responses persisted through 7 d
395 (Table 2). The 2 d EROD responses ranged from 27.5 – 59.4 pmol mg⁻¹ min⁻¹, and the 7
396 d responses ranged from 2.73 – 100 pmol mg⁻¹ min⁻¹. The EROD replicates also
397 corresponded within factors of ~ 1.5 except for the 7 d responses at the Disk Island site
398 (site 3), where the EROD response of the replicate in the stream-flushed peat was lower
399 than that of the other less well flushed replicate by a factor of ~ 6 (Springman et al.
400 2008).

401

402 The SPMDs deployed at the local control sites within the ANS sites accumulated
403 far less PAH than those deployed at the oiled sites. The TPAH concentrations ranged
404 from 132 – 299 ng g⁻¹, implying ambient seawater concentrations below ~20 ng l⁻¹,
405 consistent with results from direct measurements of background PAH in PWS seawater
406 (Boehm et al. 2007, 2005; Carls et al. 2006, Neff et al. 2006). Extracts from these
407 SPMDs consistently failed to elicit a significant EROD response following injection into
408 juvenile trout (Table 2; Springman et al. 2008).

409

410 3.3 Human Activity Sites

411

412 Although PAH were readily accumulated by SPMDs deployed within patches of
413 oil lingering from fuel tanks damaged by the 1964 Alaska earthquake, the induction of
414 hepatic CYP1A following injection of extracted contaminants into trout was more

415 variable than results from the oiled ANS sites. The patches of remnant oil at 4 of the HA
416 sites were surficial asphalt pavements, and the SPMDs deployed within them
417 accumulated TPAH concentrations ranging from 392 – 20,900 ng g⁻¹ (Table 2). The
418 distribution of PAH homologues resembled that found at the small boat harbors (compare
419 Figs. 2B and 4B), with phenanthrenes/anthracenes and fluoranthenes/pyrenes the most
420 abundant homologous series, declining abundance with increasing alkyl substitution
421 within each series, and chrysenes accounting for 0.5 – 5% of the TPAH. Following
422 injection into trout, these extracts consistently elicited low but significant 2 d EROD
423 responses ranging from 2.91 – 6.40 pmol mg⁻¹ min⁻¹ (Table 2; Springman et al. 2008).
424 These responses subsided substantially by 7 d, ranging from 1.75 – 2.74 pmol mg⁻¹ min⁻¹.

425

426 In contrast with the surficial asphalt patches at 4 of the HA sites, the SPMD at site
427 9 (at McClure Bay) was deployed in contact with the only occurrence of subsurface
428 Monterey Formation oil we have found in PWS, despite extensive sampling (Short et al.
429 2004, 2006). Chemical analysis confirmed the Monterey Formation origin of this oil,
430 which is still semi-liquid after 40 years. The distribution of PAH homologues at site 9
431 resembles those of the SPMDs deployed at the ANS sites, showing the preponderance of
432 alkyl-substituted PAH characteristic of crude and heavy refined oils, with chrysene
433 homologues accounting for 6.7% of the TPAH (Fig. 4). Although the TPAH
434 concentration in the site 9 SPMD was not the greatest among the HA deployments, the
435 combined chrysene homologues were (Table 2), and the associated EROD response was
436 also much greater at 2 d (at 37.6 pmol mg⁻¹ min⁻¹) and at 7 d (at 15.7 pmol mg⁻¹ min⁻¹).
437 These results illustrate how burial preserves CYP1A-inducing components of the oil, and

438 suggest that buried ANS oil may remain a potent source of CYP1A-inducing compounds
439 for decades, given the current slow loss rate of subsurface ANS oil from beaches in PWS.

440

441 The SPMDs deployed at the local control locations within the HA sites show that
442 the PAH contamination from the asphalt patches did not extend much beyond them. Just
443 100 m on either side of these patches, TPAH concentrations in SPMDs ranged from 41 –
444 229 ng g⁻¹, similar to results from the control locations within the ANS sites. As with the
445 ANS local controls, extracts from the HA control SPMDs consistently failed to elicit a
446 significant EROD response following injection into juvenile trout (Table 2; Springman et
447 al. 2008).

448

449 *3.4 Salmon Hatcheries*

450

451 Substantial PAH were accumulated by the SPMDs deployed at 2 of the salmon
452 hatcheries (sites 13 and 15, Table 2), only one of which induced CYP1A. The
453 deployment at the hatchery in Sawmill Bay (site 13) was near several hundred creosote
454 pilings. This SPMD accumulated a TPAH concentration of 15,200 ng g⁻¹, with a PAH
455 homologue distribution similar to those elsewhere in Sawmill Bay (compare Figs. 2B and
456 2C), except for the greater contributions from chrysenes and 5-ring PAH. The higher
457 concentrations and proportions of 4- and 5-ring PAH from site 13 resulted in a stronger 2
458 d EROD response (34.8 pmol mg⁻¹ min⁻¹) compared with extracts from the other sites
459 within Sawmill Bay (sites 2 and 44, Table 2), and this response remained elevated
460 through 7 d (at 4.90 pmol mg⁻¹ min⁻¹).

461

462 In contrast, a TPAH concentration of 10,900 ng g⁻¹ accumulated by the SPMD
463 deployed at Noerenburg hatchery (site 15) failed to elicit a significant EROD response
464 following injection into trout ($P > 0.92$ for 2 d results; Springman et al. 2008; Table 2).
465 The distribution pattern of the accumulated PAH indicates a diesel oil source, because the
466 homologue distribution is truncated beyond the phenanthrenes/anthracenes (Fig. 5),
467 reflecting the effect of distillation during refining. The failure of this extract to induce
468 CYP1A corroborates the low induction potency of lower molecular mass PAH noted
469 previously (Sundberg et al. 2005, Barron et al. 2004). Hence diesel spills, although
470 chronic in PWS as a result of marine vessel traffic associated with commercial fishing,
471 tourism, and personal transportation, may be discounted as a significant cause of CYP1A
472 induction in biota.

473

474 3.5 Salmon Streams

475

476 Concentrations of PAH accumulated by SPMDs deployed at the salmon streams
477 during spring and summer ranged from 57 – 585 ng g⁻¹ ($N = 10$; Table 2), similar to
478 ranges from other sites not impacted by obvious local sources of PAH. Despite these low
479 TPAH concentrations, there was a small but significant increase in EROD response from
480 the late summer deployments compared with those of late spring (Springman et al. 2008).
481 The salmon streams sampled ($N = 5$) exhibited a high degree of inter-site consistency in
482 spring (2 d mean value of 2.7 ± 0.062 pmol mg⁻¹ min⁻¹, CV = 0.052), which was repeated
483 for the summer sampling with consistent, slightly elevated 2 d responses ranging from

484 3.24 – 5.73. This slight seasonal increase in EROD response was attributed to
485 contaminants associated with decomposing salmon carcasses by Springman et al. (2008).

486

487 *3.6 Random Sites, Constantine Harbor and the Regional Control Site*

488

489 The TPAH concentrations of SPMDs deployed at the random sites were
490 consistently low, and showed no evidence of a seasonal difference. The TPAH
491 concentrations of the spring deployments ranged from 25 – 374 ng g⁻¹ (mean = 130 ng g⁻¹
492 ¹, $N = 31$), compared with a summer range of 40 – 442 ng g⁻¹ (mean = 126 ng g⁻¹, $N = 13$).
493 Aside from the two sites where TPAH concentrations were greatest, (sites 26 and 44,
494 discussed in sec. 3.1 above) and one site where naphthalenes account for over half the
495 PAH detected (site 33, where TPAH = 332 ng g⁻¹), the TPAH concentrations were below
496 212 ng g⁻¹. This range of concentrations was similar to those found in the SPMD field
497 blanks, where TPAH ranged from 63 – 213 ng g⁻¹, consisting mostly of naphthalenes with
498 some phenanthrenes and fluoranthenes/pyrenes detected consistently, and
499 dibenzothiophenes and chrysenes occasionally detected near MDLs. Because random
500 deployment sites may be taken as characteristic of PWS as a whole, these results imply
501 that ambient concentrations of TPAH in seawater within the Sound were usually less than
502 ~ 15 ng l⁻¹, similar to results from the local control deployments at the ANS and HA sites.
503 Collectively these results indicate that PWS is remarkably free of pollution from PAH, as
504 well as from pesticides and PCBs, when nearby sources are absent.

505

506 The SPMD extracts from the random sites failed to induce significant EROD
507 responses following injection into trout (Table 2). The 2 d EROD responses ranged from
508 1.70 – 4.78 pmol mg⁻¹ min⁻¹ (N = 9), compared with responses from field and laboratory
509 blanks that ranged from 2.80 – 6.40 pmol mg⁻¹ min⁻¹ (N = 5). This confirms that EROD
510 inducing agents were generally absent in PWS.

511

512 The low ambient PAH concentrations of PWS are probably typical of seawater in
513 the northern Gulf of Alaska generally. Comparable TPAH concentrations were found in
514 SPMDs deployed at Constantine Harbor and at the regional control site in northern
515 southeast Alaska (sites 53 and 54 respectively, Table 2). The SPMD deployed at
516 Constantine Harbor accumulated a TPAH concentration similar to those found in the field
517 blanks (Table 2), despite exposure to interstitial water draining sediments containing a
518 TPAH concentration of ~ 560 ng g⁻¹ (Short and Babcock 1996). This failure of the
519 SPMD to accumulate TPAH from these sediments demonstrates that PAH associated
520 with hydrocarbon source rock that is eroded by glaciers and then transported by the
521 Alaska Coastal Current along the northern Gulf of Alaska into PWS do not induce
522 CYP1A in fish and other biota, contrary to Huggett et al. (2003) (see Springman et al.
523 2008 for more discussion).

524

525 *3.7 Identification of CYP 1A-Inducing Compounds in PWS*

526

527 We sought to identify which components of the injected PAH mixtures are most
528 closely associated with CYP 1A induction by regression analysis. We began with a

529 comparison of linear and non-linear regressions of EROD activity on TPAH using a
530 variety of functions and ranking results according to the goodness-of-fit as determined by
531 the adjusted multiple- R^2 and model parsimony. The relation $EROD = a [TPAH]^b$ (eq. 2)
532 accounted for the variability of the data better than any other function evaluated,
533 including those with as many as four parameters (Marquardt-Levenberg non-linear
534 regression $R^2 = 0.87$, $N = 62$; Fig. 10A). When the TPAH concentration of the injection
535 mixtures is low, EROD activity is consistently at the basal level, implying that PAH are
536 the likely inducing compounds rather than some other unknown contaminants absorbed
537 by the SPMDs but not measured by our chemical analysis.

538
539 The CYP 1A induction potencies of PAH vary depending on the number of
540 aromatic rings, with 4- and 5-ring compounds much more potent than PAH with fewer
541 rings (Barron et al. 2004). We therefore compared non-linear regressions of TPAH, total
542 4- & 5-ring PAH and total chrysenes with EROD activity to identify more precisely
543 which PAH homologues in our injection mixtures best account for EROD induction.
544 Using the same 62 samples that were used above to determine the functional form for
545 EROD response to TPAH (eq. 2), the adjusted multiple- R^2 values for total 4- & 5-ring
546 PAH and total chrysenes were 0.87 and 0.92 respectively. This indicates the chrysenes
547 account best for the EROD activity for the mixtures we injected, whereas the total 4- &
548 5-ring PAH account no better than do the TPAH. The closer association of the
549 chrysenes implies that other PAH do not materially contribute to EROD induction in the
550 mixtures we injected. The regression equation for the chrysenes is $EROD = 58.3$
551 $[chrysenes]^{0.306}$. Comparison of a plot of this equation and associated data with the

552 original plot for TPAH (Fig. 6) illustrates the better fit with the chrysenes, caused mostly
553 by 3 samples that had elevated TPAH but undetectable chrysenes and low associated
554 EROD activities. Note that the functional form of the EROD response implies that
555 increasing responses imply disproportionately larger increases in exposure
556 concentrations, which should be considered when interpreting results of monitoring
557 programs based on the SPMD-extract injection method presented here.

558
559 The close association of chrysenes and EROD response is not surprising, given
560 the PAH composition of the sources we evaluated and the general absence of other
561 CYP1A-inducing compounds in PWS. Compared with the model CYP 1A inducer
562 benzo[*a*]pyrene, chrysene is more abundant in these sources (especially in ANS oil,
563 where its concentration is more than 50-fold greater), and it is about as water-soluble
564 (Mackay et al. 1992). This higher abundance offsets the lower CYP1A induction potency
565 of chrysene, which is ~25% that of benzo[*a*]pyrene (Barron et al. 2004). In addition,
566 alkyl-chrysenes are even more abundant in ANS oil and other PWS sources, although
567 considerably less water soluble. Alkyl-phenanthrenes are both abundant in PWS sources
568 and substantially more soluble than are correspondingly-substituted chrysenes (Mackay
569 et al. 1992), but their CYP1A induction potencies are lower by at least one order of
570 magnitude. Other PAH are either not as abundant or are not as potent (or both) as are the
571 chrysene homologues. We therefore conclude that chrysenes are primarily responsible
572 for CYP 1A induction in PWS, at least when exposure occurs through aquatic contact
573 (ingestion of prey contaminated by CYP 1A-inducing compounds is an independent
574 exposure route that is not assessed by the SPMD-extract injection method described

575 here). Hence, bioavailable sources containing substantial concentrations of chrysene
576 homologues are likely to stimulate CYP1A induction in appropriate test organisms
577 through aquatic exposure, and induction in resident biota exposed to these sources would
578 not be surprising. Such sources are relatively few in PWS and are limited to specific
579 anthropogenic impacts, the *Exxon Valdez* oil spill being the largest.

580

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582

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587

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754 **Tables**

755
756 Table 1. Names and abbreviations of contaminants analyzed in SPMDs.

757
758 Table 2. Concentrations of PAH, PCB and pesticides in semipermeable membrane
759 devices deployed in Prince William Sound, Alaska during 2004, and injected into
760 juvenile rainbow trout along with the consequent induction of hepatic CYP1A as
761 measured by the EROD assay (see Methods). ANS = Alaska North Slope crude oil; Chry
762 = total chrysenes; LOQ = limit of quantitation; SEAK = southeastern Alaska. Mean
763 EROD responses are from Springman et al. (2008).

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772 **Figure Legends**

773

774 Figure 1. Map of study region. Site numbers are also listed in Table 2. Dashed lines
775 indicate Alaska Department of Fish and Game districts.

776

777 Figure 2. Relative PAH concentrations accumulated by SPMDs deployed at (A) Cordova
778 harbor (site 1), (B) Chenega harbor, Sawmill Bay and Windy Bay (sites 2, 26 and 44),
779 and (C) Sawmill Bay salmon hatchery (site 13). Vertical lines indicate ranges here and
780 following. See Table 1 for PAH abbreviations.

781

782 Figure 3. Proportions of total PCB concentrations of all field SPMD samples analyzed
783 for this study ($N = 94$). Numbers on abscissa identify PCB homologues (see Table 1).

784

785 Figure 4. Relative PAH concentrations accumulated by SPMDs deployed at oiled (A)
786 ANS sites 3 – 7, (B) surface (sites 8 and 10 – 12) and (C) subsurface (site 9) Monterey
787 Formation sites.

788

789 Figure 5. Relative PAH concentrations accumulated by the SPMD deployed at site 15
790 (W. H. Noerenburg salmon hatchery on Esther Island) (site 15, Table 2). See Table 1 for
791 PAH homologue abbreviations.

792

793 Figure 6. EROD response following injection of contaminants expressed as (A) TPAH
794 and (B) total chrysenes accumulated by SPMDs into rainbow trout.

ACCEPTED MANUSCRIPT

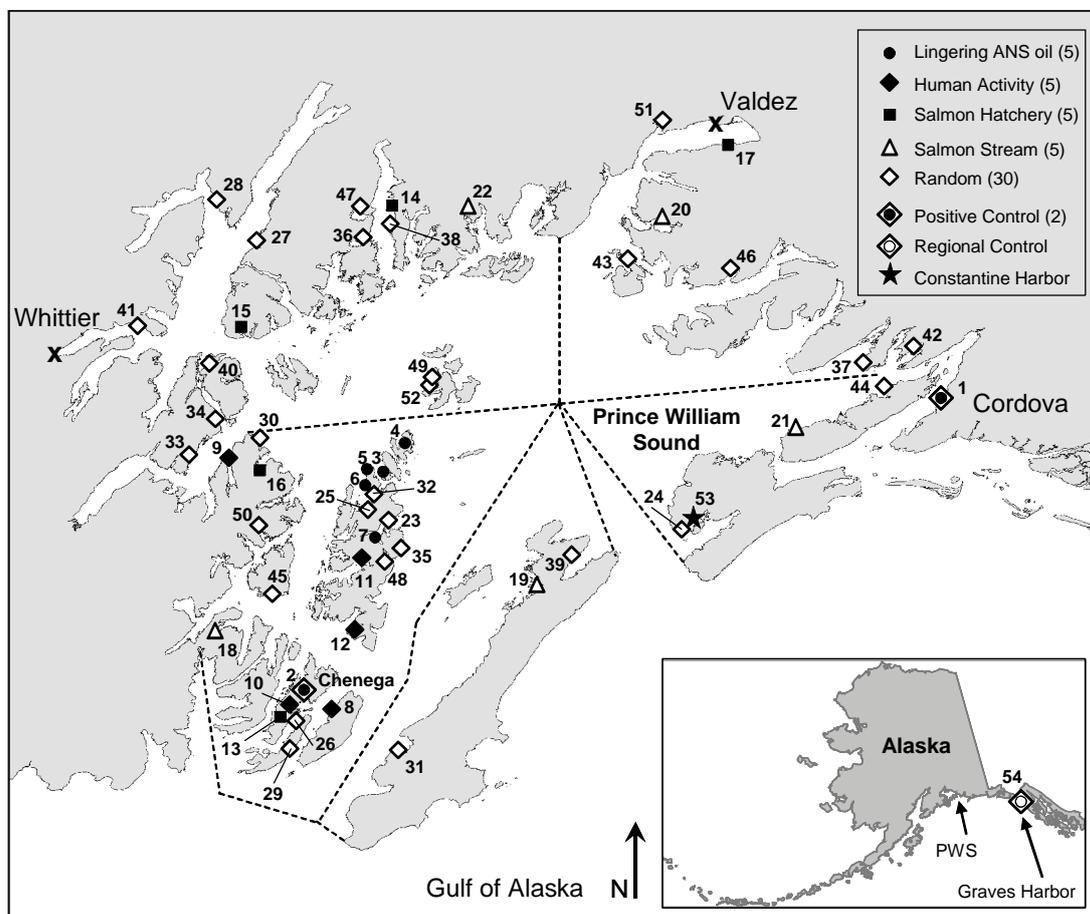


Figure 1.

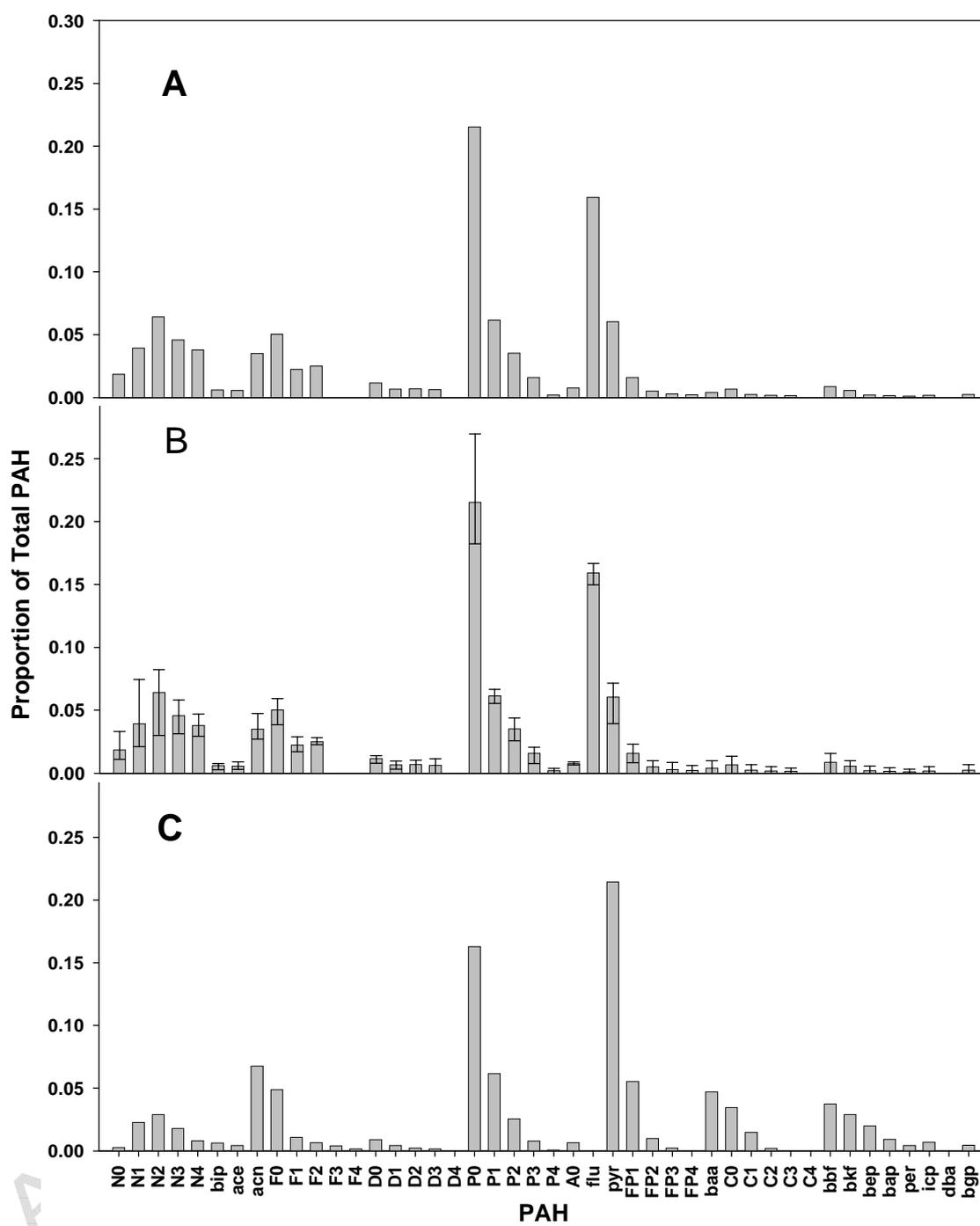


Figure 2.

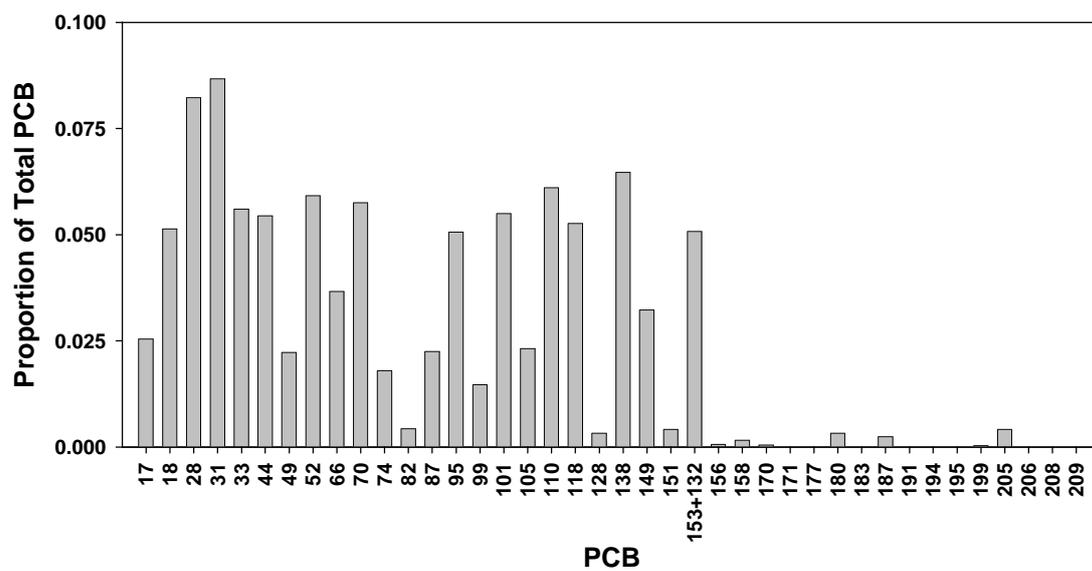


Figure 3.

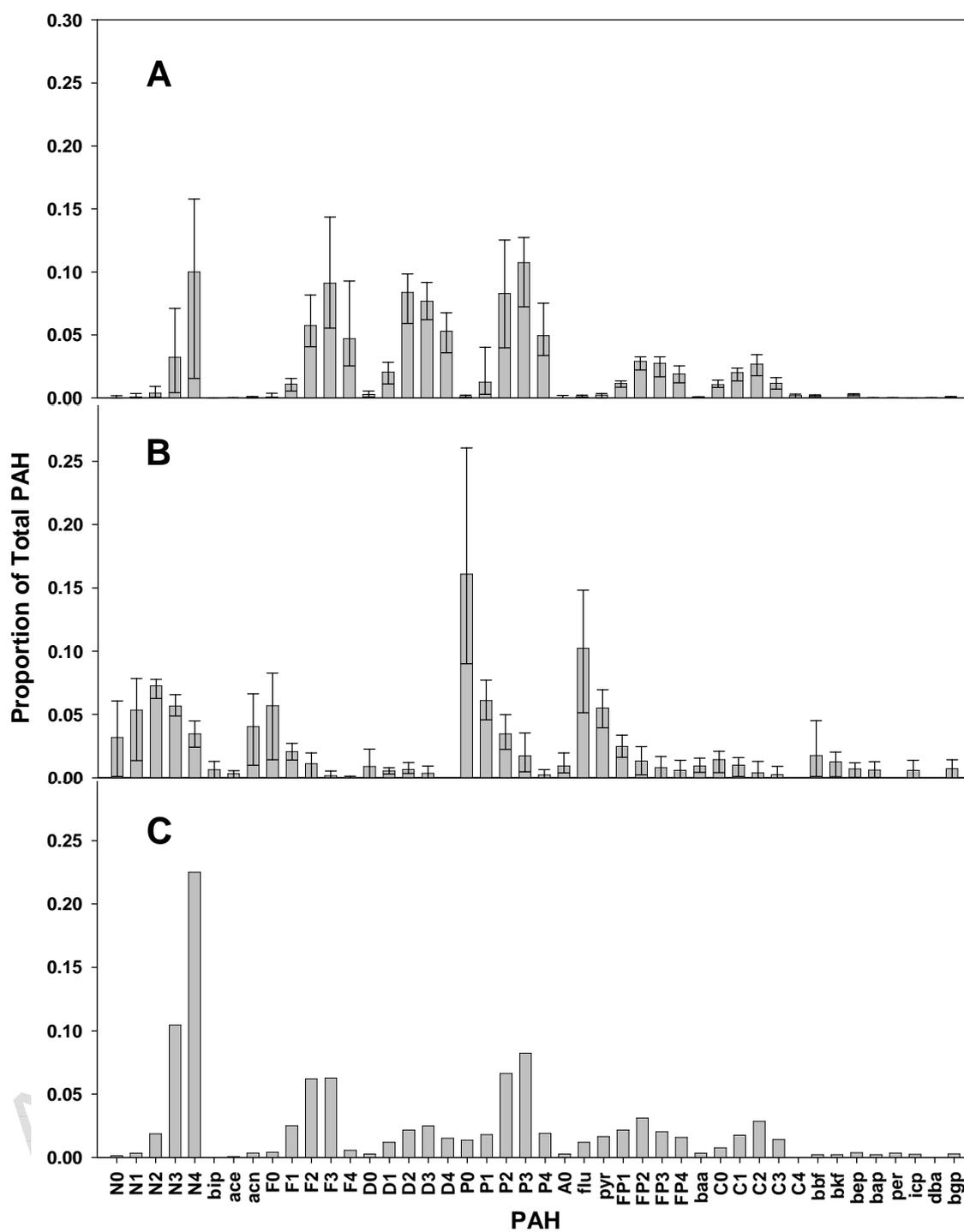


Figure 4.

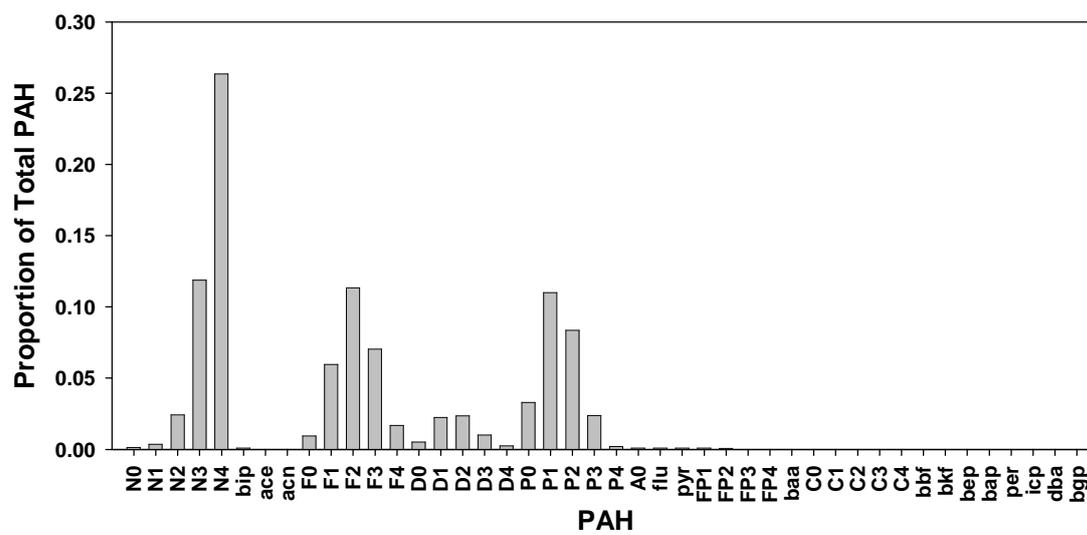


Figure 5.

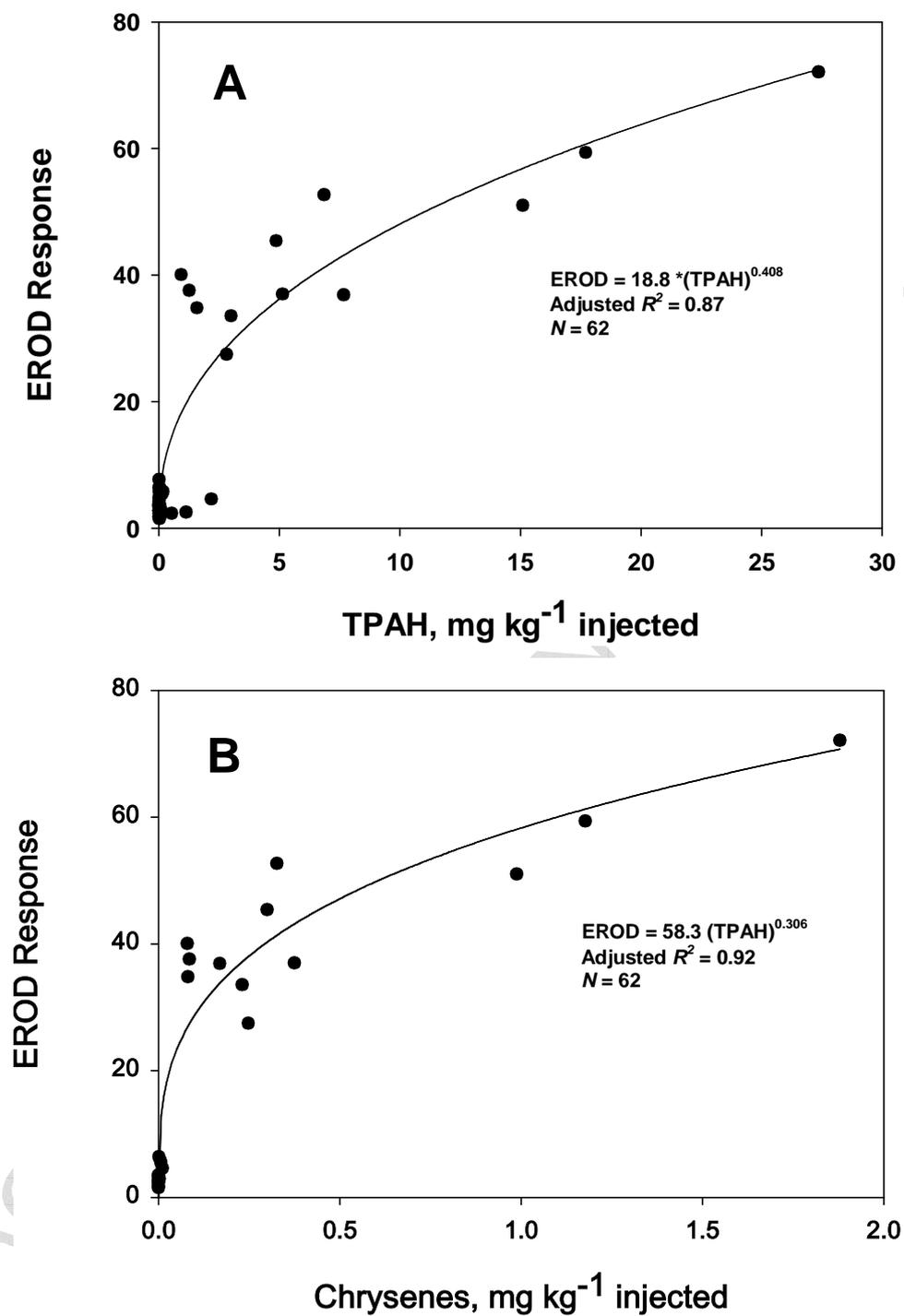


Figure 6.

Table 1.

PAH	PCB Homologue Number	PCB Number	Pesticide
Naphthalene	N0	17	Hexachlorobenzene
C1-Naphthalenes	N1	18	α -Hexachlorocyclohexane
C2-Naphthalenes	N2	28	β -Hexachlorocyclohexane
C3-Naphthalenes	N3	31	Lindane
C4-Naphthalenes	N4	33	α -Chlordane
Biphenyl	bip	44	<i>cis</i> -Nonachlor
Acenaphthene	ace	49	γ -Chlordane
Acenaphthylene	acn	52	Heptachlor
Fluorene	F0	66	Heptachlor epoxide
C1-Fluorenes	F1	70	Nonachlor III
C2-Fluorenes	F2	74	oxy-Chlordane
C3-Fluorenes	F3	82	<i>trans</i> -Nonachlor
C4-Fluorenes	F4	87	Aldrin
Dibenzothiophene	D0	95	Dieldrin
C1-Dibenzothiophenes	D1	99	Mirex
C2-Dibenzothiophene	D2	101	endo-Sulfan I
C3-Dibenzothiophene	D3	105	<i>o,p'</i> -DDD
C4-Dibenzothiophene	D4	110	<i>o,p'</i> -DDE
Phenanthrene	P0	118	<i>o,p'</i> -DDT
C1- Phenanthrenes/Anthracenes	P1	128	<i>p,p'</i> -DDD
C2- Phenanthrenes/Anthracenes	P2	138	<i>p,p'</i> -DDE
C3- Phenanthrenes/Anthracenes	P3	149	<i>p,p'</i> -DDT
C4- Phenanthrenes/Anthracenes	P4	151	
Anthracene	A0	153 + 132	
Fluoranthene	flu	156	
Pyrene	pyr	158	
C1-Fluoranthenes/Pyrenes	FP1	170	
C1-Fluoranthenes/Pyrenes	FP2	171	
C1-Fluoranthenes/Pyrenes	FP3	177	
C1-Fluoranthenes/Pyrenes	FP4	180	
Benz[<i>a</i>]anthracene	baa	183	
Chrysene	C0	187	
C1-Chrysenes	C1	191	
C2-Chrysenes	C2	194	
C3-Chrysenes	C3	195	
C4-Chrysenes	C4	199	

Benzo[<i>b</i>]fluoranthene	bbf	
Benzo[<i>k</i>]fluoranthene	bkf	205
Benzo[<i>e</i>]pyrene	bep	206
Benzo[<i>a</i>]pyrene	bap	208
Perylene	per	209
Indeno[1,2,3- <i>cd</i>]pyrene	icp	
Dibenz[<i>a,h</i>]anthracene	dba	
Benzo[<i>ghi</i>]perylene	bgp	

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Hatcheries

(13)	15,200	780	3.91	1.33	1.58	0.081	0.0004	0.0001	34.8	4.90
(14)	149	<LOQ	2.84	2.37	0.02	<LOQ	0.0003	0.0002	1.99	1.49
(15)	10,900	<LOQ	3.64	1.02	1.13	<LOQ	0.0004	0.0001	2.53	2.15
(16)	329	<LOQ	2.93	4.39	0.03	<LOQ	0.0003	0.0005	4.88	1.55
(17)	233	<LOQ	5.51	2.88	0.02	<LOQ	0.0006	0.0003	2.53	2.00

Salmon

Streams

Spring

(18)	211	<LOQ	2.93	3.03	0.022	<LOQ	0.0003	0.0003	2.47	1.41
(19)	585	<LOQ	2.22	0.94	0.061	<LOQ	0.0002	0.0001	2.68	0.98
(20)	150	<LOQ	2.76	2.74	0.016	<LOQ	0.0003	0.0003	2.75	1.48
(21)	168	<LOQ	2.67	2.61	0.017	<LOQ	0.0003	0.0003	2.84	1.71
(22)	191	<LOQ	4.71	3.32	0.020	<LOQ	0.0005	0.0003	2.60	1.52

Salmon

Streams

Summer

(18)	57	<LOQ	3.11	2.76	0.006	<LOQ	0.0003	0.0003	3.48	1.13
(19)	99	<LOQ	1.87	1.79	0.010	<LOQ	0.0002	0.0002	3.24	1.53
(20)	125	<LOQ	7.02	4.33	0.013	<LOQ	0.0007	0.0004	5.73	4.79
(21)	103	<LOQ	2.49	2.78	0.011	<LOQ	0.0003	0.0003	3.96	3.00
(22)	107	<LOQ	6.67	3.02	0.011	<LOQ	0.0007	0.0003	3.60	1.76

Random

Sites

Spring

(23)	179	<LOQ	8.89	3.82	0.019	<LOQ	0.0009	0.0004	2.67	0.59
(24)	211	<LOQ	15.1	3.41	0.022	<LOQ	0.0016	0.0004	1.98	1.76
(25)	205	<LOQ	3.38	3.30	0.021	<LOQ	0.0004	0.0003	2.97	0.65
(26)	374	2	41.8	5.52	0.039	<LOQ	0.0043	0.0006	3.56	1.48
(27)	79	<LOQ	2.67	3.98						
(28)	49	<LOQ	2.49	3.60						
(29)	59	<LOQ	1.24	1.21						
(30)	33	<LOQ	2.09	3.41						
(31)	110	<LOQ	<LOQ	2.67						
(32)	113	<LOQ	0.68	0.89						
(33)	322	<LOQ	1.73	2.49						
(34)	93	<LOQ	2.40	3.52						
(35)	25	<LOQ	2.18	3.31						
(36)	70	<LOQ	1.78	2.50						
(37)	69	<LOQ	2.04	3.32						
(38)	35	<LOQ	2.44	2.43						
(39)	29	<LOQ	2.67	3.59						

Random

Sites

Summer

(40)	102	<LOQ	2.22	1.62	0.011	<LOQ	0.0002	0.0002	1.81	1.86
(41)	128	<LOQ	2.13	3.30	0.013	<LOQ	0.0002	0.0003	2.38	1.70
(42)	127	<LOQ	3.56	3.13	0.013	<LOQ	0.0004	0.0003	3.10	1.64
(43)	77	<LOQ	4.00	2.75	0.008	<LOQ	0.0004	0.0003	4.78	0.98
(44)	442	<LOQ	6.13	1.72	0.046	<LOQ	0.0006	0.0002	1.70	1.97
(45)	91	<LOQ	1.56	3.03						
(46)	48	<LOQ	2.49	2.82						

(47)	56	<LOQ	1.96	2.39						
(48)	71	<LOQ	2.49	3.25						
(49)	40	<LOQ	1.87	2.75						
(50)	138	<LOQ	4.31	2.78						
(51)	208	<LOQ	2.18	2.25						
(52)	104	<LOQ	5.78	3.96						
Constantine Harbor										
(53)	197	<LOQ	2.93	3.48	0.020	<LOQ	0.0003	0.0004	2.83	1.39
Regional Control SEAK										
(54)	258	<LOQ	3.11	4.61	0.027	<LOQ	0.0003	0.0005	2.59	1.87
Field Blanks										
(18)	109	<LOQ	2.31	1.35	0.011	<LOQ	0.0002	0.0001	3.80	1.61
(42)	132	<LOQ	16.3	1.05	0.008	<LOQ	0.0010	0.0001	3.57	4.82
	213	3	3.41	1.07						
	88	<LOQ	3.20	0.08						
	63	<LOQ	2.80	2.74						
(27)	127	<LOQ	2.00	1.56						
	120	3	2.84	4.73						
	83	<LOQ	2.76	2.11						
Lab Blanks										
	89	<LOQ	<LOQ	2.92	0.009	<LOQ	<LOQ	0.0003	2.80	1.17
	71	<LOQ	<LOQ	0.31	0.007	<LOQ	<LOQ	<LOQ	6.40	5.02
	63	<LOQ	0.40	0.58	0.001	<LOQ	<LOQ	<LOQ	2.80	1.17
	0	<LOQ	<LOQ	0.03						
	18	<LOQ	<LOQ	0.32						